From: Dave Russell/ESC/R3/USEPA/US

Sent: 2/23/2012 4:21:46 PM

To: Cynthia Caporale/ESC/R3/USEPA/US@EPA

CC:

Subject: Re: HT for Micro

The holding time for a micro sample always depends on the analyte and the rule under which the samples are being collected.

The HT for total coliforms, fecals or E. coli is **30 hours** under the TCR (TC, FC, E.coli) and the GWR (E. coli), **8 hours** under the SWTR (TC,FC), and **6 hours** under NPDES (TC,FC,E.coli). When reviewing Dimock data for TC/FC I have applied the **30 hour** holding time, and thus far, <u>all samples have met that holding time</u>.

The HT for heterotrophic plate count is **8 hours** under the SWTR. HPC is only used as an indicator under the SWTR. The **8-hour** holding time for HPC is stated in the SDWA Lab Cert Manual and twice in Standard Methods. For Dimock data I have applied the **8 hour** HT, and although many samples have not met this HT, the result has always been to qualify the data as an <u>estimate</u> and as such it can still be used (with a flag/qualifier). <u>No HPC results have been rejected based on holding time.</u>

In Standard Methods, the **8-hour** HT is given at 9060 B(1)b: "Do not exceed 8 h holding time for heterotrophic plate counts.", [clear and emphatic] and a second time at 9215 A(4): "The recommended maximum elapsed time between collection and analysis of samples is 8 h (maximum transit time 6 h, maximum processing time 2 h). When analysis cannot begin within 8 h, maintain sample at a temperature below 4C but do not freeze. Maximum elapsed time between collection and analysis must not exceed 24 h."

The two papers on bacteriological holding time published by EPA microbiologists in Cincinnati indicate that the bacterial community in a sample can begin to change as soon as the sample is collected. Bacterial abundance may increase, decrease or remain the same. Those findings make the last resort 24-hour option offered by SM rather indefensible, and even if the 24-hour HT was applied to Dimock HPC data, the samples were not held below 4C following collection.

Hope this answers the question.

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